CLAIMS

We claim:

1. A method for detecting a first target nucleic acid sequence comprising:

a) hybridizing at least a first primer nucleic acid to said first target sequence to form a first hybridization complex;

b) contacting said first hybridization complex with a first enzyme to form a modified first primer nucleic acid;

c) disassociating said first hybridization complex;

d) contacting said modified first primer nucleic acid with an array comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first subpopulation comprising a first capture probe; such that said first capture probe and the modified primer form an assay complex; wherein said microspheres are distributed on said surface; and

e) detecting the presence of the modified primer nucleic acid.

2. A method according to claim 1 wherein steps a) through c) are repeated prior to step d).

3. A method according to claim 1 further comprising:

a) hybridizing at least a second primer nucleic acid to a second target sequence that is substantially complementary to said first target sequence to form a second hybridization complex;

b) contacting said second hybridization complex with said first enzyme to form a modified second primer nucleic acid;

c) disassociating said second hybridization complex; and

d) forming a second assay complex comprising said modified second primer nucleic acid and a second capture probe on a second subpopulation.

4. A method according to claim 3 wherein steps a) through c) are repeated prior to step d).

5. A method according to claim 2 wherein said first enzyme comprises a DNA polymerase and said modification is an extension of said primer such that the polymerase chain reaction (PCR) occurs.

6. A method according to claim 2 wherein said first enzyme comprises a ligase and said modification comprises a ligation of said first primer which hybridizes to a first domain of said first target sequence to a third primer which hybridizes to a second adjacent domain of said first target sequence, such that the ligase chain reaction (LCR) occurs.

7. A method according to claim 3 wherein said first enzyme comprises a ligase and said modification

is a ligation of said second primer which hybridizes to a first domain of said second target sequence to a fourth primer which hybridizes to a second adjacent domain of said second target sequence, such that the ligase chain reaction (LCR) occurs.

8. A method according to claim 2 wherein said first primer comprises a first probe sequence, a first scissile linkage and a second probe sequence, wherein said first enzyme will cleave said first scissile linkage resulting in the separation of said first and said second probe sequences and the disassociation of said first hybridization complex, leaving said first target sequence intact, such that the cycling probe technology (CPT) reaction occurs.

9. A method according to claim 4 wherein said second primer comprises a third probe sequence, a second scissile linkage and a fourth probe sequence, wherein said first enzyme will cleave said second scissile linkage resulting in the separation of said third and said fourth probe sequences and the disassociation of said second hybridization complex, leaving said second target sequence intact, such that the cycling probe technology (CPT) reaction occurs.

10. A method according to claim 2 wherein said first enzyme is a polymerase that extends said first primer and said modified first primer comprises a first newly synthesized strand, and said method further comprises:

a) the addition of a second enzyme comprising a nicking enzyme that nicks said extended first primer leaving said first target sequence intact; and

b) extending from said nick using said polymerase, thereby displacing said first newly synthesized strand and generating a second newly synthesized strand;

such that strand displacement amplification (SDA) occurs.

11. A method according to claim 4 wherein said first enzyme is a polymerase that extends said second primer and said modified first primer comprises a third newly synthesized strand, and said method further comprises:

a) the addition of a second enzyme comprising a nicking enzyme that nicks said extended second primer leaving said second target sequence intact; and

b) extending from said nick using said polymerase, thereby displacing said third newly synthesized strand and generating a fourth newly synthesized strand;

such that strand displacement amplification (SDA) occurs.

12. A method according to claim 2 wherein said first target sequence is a RNA target sequence, said first primer nucleic acid is a DNA primer comprising an RNA polymerase promoter, said first enzyme is a reverse-transcriptase that extends said first primer to form a first newly synthesized DNA strand, and said method further comprises:

a) the addition of a second enzyme comprising an RNA degrading enzyme that degrades said

first target sequence;

b) the addition of a third primer that hybridizes to said first newly synthesized DNA strand;

c) the addition of a third enzyme comprising a DNA polymerase that extends said third primer to form a second newly synthesized DNA strand, to form a newly synthesized DNA hybrid;

d) the addition of a fourth enzyme comprising an RNA polymerase that recognizes said RNA polymerase promoter and generates at least one newly synthesized RNA strand from said DNA hybrid;

such that nucleic acid sequence-based amplification (NASBA) occurs.

13. A method according to claim 2 wherein said first primer is an invader primer, said method further comprises hybridizing a signalling primer to said target sequence, said enzyme comprises a structure-specific cleaving enzyme and said modification comprises a cleavage of said signalling primer, such that the invasive cleavage reaction occurs.

14. A method for detecting a target nucleic acid sequence comprising:

a) hybridizing a first primer to a first target sequence to form a first hybridization complex;

b) contacting said first hybridization complex with a first enzyme to extend said first primer to form a first newly synthesized strand and form a nucleic acid hybrid that comprises an RNA polymerase promoter;

c) contacting said hybrid with an RNA polymerase that recognizes said RNA polymerase promoter and generates at least one newly synthesized RNA strand;

d) contacting said newly synthesized RNA strand with an array comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first subpopulation comprising a first capture probe; such that said first capture probe and the modified primer form an assay complex; wherein said microspheres are distributed on said surface; and

e) detecting the presence of the newly synthesized RNA strand.

15. A method according to claim 14 wherein steps a) through c) are repeated prior to step d).

16. A method according to claim 14 wherein said target nucleic acid sequence is a RNA sequence, and prior to step a), said method comprises:

f) hybridizing a second primer comprising an RNA polymerase promoter sequence to said RNA sequence to form a second hybridization complex;

g) contacting said second hybridization complex with a second enzyme to extend said second primer to form a second newly synthesized strand and form a nucleic acid hybrid; and

h) degrading said RNA sequence to leave said second newly synthesized strand as said first target sequence.

17. A method according to claim 16 wherein said degrading is done by the addition of an RNA degrading enzyme.

18. A method according to claim 16 wherein said degrading is done by RNA degrading activity of said reverse transcriptase.

19. A method according to claim 14 wherein said target nucleic acid sequence is a DNA sequence, and prior to step a), said method comprises:

f) hybridizing a second primer comprising an RNA polymerase promoter sequence to said DNA sequence to form a second hybridization complex;

g) contacting said second hybridization complex with a second enzyme to extend said second primer to form a second newly synthesized strand and form a nucleic acid hybrid; and

h) denaturing said nucleic acid hybrid such that said second newly synthesized strand is said first target sequence.

20. A method according to claim 1 wherein said first primer nucleic acid hybridizes at its 5' end to said target nucleic acid sequence and at its 3' end to a sequence immediately adjacent to said 5' end, wherein said first enzyme comprises a ligase and said modification comprises ligation of said 5' end with said 3' end to form a circular probe, wherein said second enzyme is a polymerase and said amplification is an amplification of said circular probe such that rolling circle amplification occurs.

21. A kit for the detection of a first target nucleic acid sequence comprising:

a) at least a first nucleic acid primer substantially complementary to at least a first domain of said target sequence;

b) at least a first enzyme that will modify said first nucleic acid primer; and

c) an array comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent; wherein said microspheres are distributed on said surface.

22. A kit according to claim 21 for the detection of a PCR reaction wherein said first enzyme is a thermostable DNA polymerase.

23. A kit according to claim 21 for the detection of a LCR reaction wherein said first enzyme is a ligase and said kit comprises a first nucleic acid primer substantially complementary to a first domain of said first target sequence and a third nucleic acid primer substantially complementary to a second adjacent domain of said first target sequence.

24. A kit according to claim 21 for the detection of a strand displacement amplification (SDA) reaction wherein said first enzyme is a polymerase and said kit further comprises a nicking enzyme.

25. A kit according to claim 21 for the detection of a NASBA reaction wherein said first enzyme is a reverse transcriptase, and said kit comprises a second enzyme comprising an RNA degrading enzyme, a third primer, a third enzyme comprising a DNA polymerase and a fourth enzyme comprising an RNA polymerase.

26. A kit according to claim 21 for the detection of an invasive cleavage reaction wherein said first enzyme is a structure-specific cleaving enzyme, and said kit comprises a signalling primer.

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